

CLAIMS

1. A plurality of polynucleotides encoding a Fab library comprising a plurality of vector wherein the vector comprises:

- 5           - a first and second cloning region, wherein
- each cloning region comprises at least one, for the vector unique, restriction enzyme cleavage site,
- 10           - each cloning region being 5' flanked by a ribosome binding site and a signal sequence,
- a polynucleotide encoding an anchor region, located 3' of the second cloning region,
- 15           - a first and a second plurality of variable polynucleotides,
- each encoding a complete antibody variable region or part of an antibody variable region, possibly followed by a complete antibody constant region or part of an antibody constant region,
- 20           - the first plurality of variable polynucleotides being cloned into the vector at the restriction enzyme cleavage site(s) of the first cloning region,
- 25

- the second plurality of variable polynucleotides being cloned into the vector at the restriction enzyme cleavage site(s) of the second cloning region.

5           2. Polynucleotide according to claim 1, wherein the first plurality of variable polynucleotides are  $V_L$  polynucleotides, and the second plurality of variable polynucleotides are  $V_H$  polynucleotides.

10           3. Polynucleotides according to any of the preceding claims, wherein the polynucleotides encode at least  $10^9$  different Fabs, preferably at least  $10^{10}$  different Fabs, most preferably at least  $3.7 \times 10^{10}$  different Fabs.

4. Fab library, comprising:

15           - a plurality of vectors, wherein the vector comprises:

- a first and a second cloning region, wherein

20           - each cloning region comprises at least one, for the vector unique, restriction enzyme cleavage site,

- each cloning region being 5' flanked by a ribosome binding site and a signal sequence,

- a polynucleotide encoding an anchor region, located 3' of the second cloning region,

- a first and a second plurality of variable polynucleotides,

5           - each encoding a complete antibody variable region or part of an antibody variable region, possibly followed by a complete antibody constant region or part of an antibody constant region,

10          - the first plurality of variable polynucleotides being cloned into the vector at the restriction enzyme cleavage site(s) of the first cloning region,

15          - the second plurality of variable polynucleotides being cloned into the vector at the restriction enzyme cleavage site(s) of the second cloning region to form a plurality of fusion polynucleotides encoding a  
20          plurality of fusion proteins,

25          - a plurality of capsid particles, wherein the plurality of vector containing the first and second pluralities of variable polynucleotides is packaged into the capsid particles, wherein

            - at least some of the capsid particles display the fusion protein encoded by the vector packaged into the capsid on the surface of the capsid.

5. Method of making a plurality of polynucleotides encoding a Fab library, comprising the steps of:

- 5       - amplifying a first plurality of variable polynucleotides with a first set of primers,
- amplifying a second plurality of variable polynucleotides with a second set of primers,
  - 10           - wherein each set of primers comprises oligonucleotides designed to be homologous to the 5' and 3' end of variable polynucleotides encoding antibody variable regions or parts thereof, such that they can be used to amplify variable polynucleotide pools from natural or synthetic sources of genes while
  - 15           retaining all or part of the antibody's antigen combining site;
- cloning the first and second plurality of variable polynucleotides into a plurality of
- 20       vectors,
  - wherein the vector comprises:
    - a first and a second cloning region, wherein
    - 25           - each cloning region comprises at least one, for the vector unique, restriction enzyme cleavage site,

- each cloning region being 5'  
flanked by a ribosome binding site  
and a signal sequence,

5                   - a polynucleotide encoding an anchor  
region, located 3' of the second cloning  
region,

10                   - wherein the first plurality of variable  
polynucleotides is cloned into the  
restriction enzyme cleavage site(s) of the  
first cloning region of the vector and the  
second plurality of variable polynucleotides  
15                   into the restriction enzyme cleavage site(s)  
of the second cloning region of the vector.

6. Method according to claim 5, further  
comprising the steps of:

20                   - cloning the second plurality of variable  
polynucleotides into a plurality of another  
vector, and excising the variable polynucleotides  
from the vector with a restriction enzyme.

7. Method of making a Fab library, wherein the  
plurality of vector containing the first and second  
25                   pluralities of variable polynucleotides, obtained  
according to claim 4 or 5, are packaged into a  
plurality of capsid particles.

8. Method for obtaining a Fab clone with specificity to a target, comprising the steps of:

- obtaining a library of claim 4, and

5                   - selecting an antigen-binding Fab using in vitro selection on immobilised or labeled antigen.

9. Monoclonal Fab or polyclonal collection of Fab clones comprising :

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- one clone, respectively a plurality of clones obtained from a library of claim 4 that specifically bind(s) to the human glycoprotein polymorphic epithelial Mucin-1 (MUC1).

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10. Vector as defined in any of the claims 1-3.